Anion Channels

2781-Pos Board B551
Allosteric Coupling Between the CFTR Chloride Channel's NBD1 Heterodimer Interface and Intracellular Domains
Jennifer Dawson, Julie Forman-Kay.
Hospital for Sick Children, Toronto, ON, Canada.
Cystic fibrosis is caused by mutations in the chloride channel CFTR, leading to loss of function and changes in the ion and fluid flow across epithelial surfaces. Like ABC transporters, CFTR contains two membrane spanning domains (MSDs) and two cytoplasmic nucleotide binding domains (NBDs). The formation of an NBD1/NBD2 dimer drives channel opening. The coupling helices at the base of the intracellular domains (ICLs) couple the NBDs to the MSDs of the channel. How are changes on the heterodimer interface transmitted across NBD1 to ICLs? The sensitivity of NMR spectroscopy reveals how the ICL4 binding site of NBD1 is allosterically linked to its heterodimer interface. During titrations, an ICL4 ''coupling helix'' peptide bound near the alpha-subdomain of NBD1, leading to destabilization and release of the C-terminal NBD1 helices 8 and 9 (H8/H9) from the heterodimer interface via an allosteric mechanism. Therefore, perturbations in one region should cause a reciprocal change in the other region. DelF508, a CF-causing mutation in the alpha-subdomain, reduces the effects of ICL4 binding on H8/H9. In contrast, DelF508-suppressor mutations, F494N and V510D, increase these effects. Helix 8 mutation, Q637R (also a DelF508-suppressor), increases the binding effects in the ICL4 binding site. Q637R also alters the dynamics in this region, suggesting that the internal motions of NBD1 are involved in transmitting changes across this plastic domain. The destabilization and release of H8/H9 from the heterodimer interface is strikingly similar to that of the regulatory extension (RE) and R region, which follow helix 9 and become more disordered and less bound to NBD1 upon phosphorylation. The RE and R region regulate NBD dimerization and, ultimately, channel opening and closing. The allosteric pathway provides insight into how dimerization may be communicated to the rest of CFTR.

2782-Pos Board B552
A Homology-Based Molecular Model of the Closed State of Human CFTR
Kazi Shefaet Rahman[1], Guiying Cui[2], Nael A. McCarty[2], Stephen C. Harvey[1].
[1]Petit Institute of Bioengineering and School of Biology, Georgia Institute of Technology, Atlanta, GA, USA, [2]Emory+Children's Center for CF Research and Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA.
Cystic fibrosis transmembrane conductance regulator (CFTR) is an ATP-gated chloride channel belonging to the ATP-binding cassette (ABC) transporter superfamily of proteins, and the locus of the primary defect in cystic fibrosis (CF). Advances in CF therapeutic research have been hampered by the lack of information about the conformational changes that CFTR undergoes during its gating cycle. Here, we present an all-atom structural model of the inward-facing, ATP-free conformation of CFTR that is based on comparative/homology modeling using as a template the experimental structure of P-glycoprotein, a closely-related ABC transporter. We believe that this model, which is supported by experimental data, corresponds to the closed state of the CFTR channel, and it provides vital clues as to what makes CFTR unique as the only member of the ABC transporter superfamily that bears channel activity. Notably, the proposed structure is significantly different from prior homology models of nucleotide-bound CFTR, which have been proposed to represent the putative open state conformation. This homology model provides the basis for examining possible pathways as the CFTR structure moves between the closed and open states, which should lead to a better understanding of the relationship between molecular structure and channel function in CFTR.
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Separation and Accessibility of the Nucleotide-Binding Domain (NBD) Heterodimerization Interface When a CFTR Channel Closes
Luiz A. Poletto Chaves, David C. Gadsby.
The Rockefeller University, New York, NY, USA.
CFTR channel gating is driven by cyclic formation and dissociation of a head-to-tail NBD1-NBD2 heterodimer enclosing two composite ATP sites, one catalytically active and one dead. NBD separation at the active site is believed to follow ATP hydrolysis there that triggers channel closure. But whether the NBDs also separate at the dead site each cycle remains unclear. To address this we determined, in patches from *Xenopus* oocytes, gating-state-dependent accessibility to MTS reagents of interfacial target cysteines in CFTR channels with one (C832S), or eight (C832-1458S), native Cys replaced by Ser. Target S549C (S in NBD1 LSGGQ) in the catalytically-active site was rapidly modified, diminishing CFTR current ($\tau_{MTS} \leq 2$ s), by micromolar MTSACE, $MTSET^+$, or $MTSES^-$ applied during channel opening and closing in 3 mM ATP. Similarly rapid current diminution attended modification, in 3 mM ATP, of the equivalent target in the catalytically-dead site, S1347C (S in NBD2 LSHGH). For both S549C and S1347C, the current decay time course upon MTS modification in ATP (τ_{MTS}) matched that of channel closure on ATP washout ($\tau_{washout}$), implying that modification occurred as soon as channels closed. Supporting that interpretation, τ_{MTS} and $\tau_{washout}$ were both slowed ~10-fold in S1347C channels bearing the hydrolysis-impairing mutation K1250R, suggesting S1347C cannot be modified in open CFTR channels. Hence, in open CFTR channels, a tight heterodimer interface in the catalytically-dead site prevents MTS access to S1347C, but NBD1-NBD2 separation allows access when channels close. Rapid modification of S549C and S1347C in 3 mM ATP by larger reagents, MTS-glucose (~14Å x 9Å x 9Å), MTS-biotin (~15Å x 11Å x 8Å), and MTS-rhodamine (~17Å x 16Å x 11Å), suggests that active and dead sites simultaneously separate by ≥11Å each gating cycle. [DK51767].

2784-Pos Board B554
On the Coupling Mechanism of CFTR Gating by ATP Hydrolysis
Kangyang Jih, Tzyh-Chang Hwang.
University of Missouri-Columbia, Columbia, MO, USA.
Once phosphorylated, CFTR, a member of the ABC protein superfamily, functions as an ATP-gated chloride channel. It is generally held that ATP-induced NBD dimerization opens the gate located in the transmembrane domains (TMDs) and subsequent ATP hydrolysis in composite site 2 causes the NBD dimer to separate partially and subsequently closes the gate. Using pyrophosphate (PPi) or AMP-PNP as a bait, we have previously identified a stable post-hydrolytic closed state (C2 state), which can be locked-open by these non-hydrolyzable analogs with a slow rate. Here we captured, upon channel closing, another novel state, which distinguishes itself by its prompt response to PPi or AMP-PNP. Nonetheless, the locked-open time from this newly identified state is no different from that of locked-open channels from the C2 state, suggesting an identical locked-open configuration. Single-channel ligand-exchange experiments revealed an open-to-locked-open transition indicating that this new state with a vacated composite site 2 is an open state (O2 state). Although a [ATP]-dependent open time was not observed with wild-type CFTR due to a limited signal noise ratio, the open time for a conserved mutant, W401F-CFTR, increases with increasing [ATP] suggesting that ATP can also bind to the O2 state and go through another hydrolysis reaction within an opening burst - thus a violation of one-to-one stoichiometry between the gating cycle and the ATP hydrolysis cycle. Interestingly, for both WT- and W401F-CFTR, experimental data as well as computer simulations based on the new gating scheme we proposed show a bimodal distribution of the open time histograms with a paucity of short events. In conclusion, our results suggest that the gating signal is transmitted from NBDs to the gate with a delay and that TMDs and NBDs do not move synchronously during a gating cycle.

2785-Pos Board B555
Declaration of Independence by CFTR's Transmembrane Domains
Xiaolong Gao, Yonghong Bai, Tzyh-Chang Hwang.
University of Missouri-Columbia, Columbia, MO, USA.
Cystic Fibrosis Transmembrane conductance Regulator (CFTR) is a unique member of the ATP-binding cassette (ABC) transporter superfamily in that it functions as an ATP-gated chloride channel. Recent cysteine scanning studies in our lab demonstrate that both the sixth transmembrane segment (TM6) and its C-terminal counterpart, TM12, play pivotal roles in chloride permeation and are also involved in gating conformational changes. Here, to study the functional role of TM1 in gating and permeation, we introduced cysteine residues into this TM and assessed their reactivity towards internally-applied thiol-directed methanethiosulfonate (MTS) reagents. Our initial cysteine scanning experiments (1-2 patches for each cysteine-substituted cysless-CFTR channels) identified four positive hits, including E92, K95, Q98 and L102, where the negatively-charged MTSES reacting with engineered cysteines diminishes ATP-induced current. Surprisingly, according to the CFTR topology based on hydropathy plots, all these positions reside in the external half of TM1- contrary to results with TM6 and TM12. We also found that modification by the positively-charged MTSET enhances macroscopic current in K95C-, Q98C-, and L102C-cysless-CFTR channels. Intriguingly, at the single-channel level, we observed that whereas the mutation L102C appears to destabilize the gate, deposition of the MTSET adduct decreases the single-channel current amplitude but increases the Po. Moreover, channel gating persisted even after

a complete removal of ATP, raising the possibility that gating motion in the TMDs of modified L102C channels is independent of that in their NBDs. Finally, MTSET modification of L102C is state-dependent, meaning that L102C-cysless-CFTR channels do not respond to the treatment of MTSET in the absence of ATP. These findings suggest that either position 102 moves during gating or gating motion in other regions of CFTR alters its reactivity towards MTSET. Overall, our preliminary data reveal several interesting yet enigmatic aspects of TM1 that call for further studies.

2786-Pos Board B556

Three Charged Amino Acids in the Outer Vestibule of CFTR Stabilize the Open Pore Architecture

Guiying Cui, Christopher Kuang, Chengyu Z. Prince, Nael A. McCarty.
Emory University, Atlanta, GA, USA.

Cystic fibrosis transmembrane conductance regulator (CFTR) carries 6 extracellular loops (ECL1-6). ECL4 bears N-linked oligosaccharide chains while the functions of other ECLs remain unknown. Few charged amino acids of ECL1 have been identified as sites of CF disease mutation, including R117C/G/P/H/L, D110H/Y/N/E, and E116K/Q. It was reported that D110H-, E116K-, and R117C/L/P-CFTR possibly impair channel stability but not R117H. We asked whether these amino acids are directly involved in ion conduction and permeation of CFTR or contribute to stabilizing the outer vestibule architecture. We used cRNA injected oocytes combined with electrophysiological technique to probe the possible function of these amino acids. We found that: (1) Mutants R117A-, D110R-, and E116R-CFTR exhibited multiple open states with significantly shortened burst duration compared with WT-CFTR, while charge-retaining mutants R117K-, D110E-, and E116D-CFTR showed mainly the full open state which rescued the open burst duration similar to WT-CFTR; (2) R117A-, D110R-, and E116R-CFTR unlike WT-CFTR failed to be locked into the open state by AMP-PNP; (3) The function of R117C-, D110C-, and E116C-CFTR were not modified by extracellular $MTSES^-$ or $MTSET^+$; (4) R117C-, D110C-, and E116C-CFTR were weakly blocked by GlyH-101 compared to WT-CFTR, while GlyH-101 strongly blocked T338A- and R352A-CFTR and completely lost its effect on R334C- and R334A-CFTR. R334, T338, and R352 are amino acids in TM6 which is the most important TM that determines ion permeation in CFTR. The data so far suggest that: (1) R117, D110, and E116 are not involved in ion conduction and permeation of CFTR directly; (2) The three charged amino acids contribute to stabilizing the CFTR channel pore; (3) The three charged amino acids probably interact with their partners to help maintain CFTR's outer vestibule architecture. (NIH-R01-DK056481).

2787-Pos Board B557

Effects of Genistein and Curcumin on Non ATP-Hydrolytic CFTR Mutants

Yumi Nakamura[1], Akiko Hanyuda[1], Ying-Chun Yu[1],
Tomoka Hagiya-Furukawa[2], Mitsuhiko Odera[2], Tzyh-Chang Hwang[3],
Minoru Sakurai[2], Masato Yasui[1], **Yoshiro Sohma**[1].
[1]Keio University School of Medicine, Tokyo, Japan, [2]Tokyo Institute of Technology, Tokyo, Japan, [3]University of Missouri - Columbia, Columbia, MO, USA.

The Cystic Fibrosis Transmembrane conductance Regulator (CFTR) chloride channel plays an important role in salt and water transport across epithelia and defective function due to mutations in the CFTR gene cause cystic fibrosis (CF). Numerous small molecules have been shown to increase the activity of CFTR mutants presumably by binding to the CFTR protein. Among the many CFTR potentiators, genistein is perhaps the most extensively studied. Recently a component of the spice turmeric, curcumin was reported to strongly activate wild type and mutated CFTR including F508del and G551D mutations. Recently we found that genistein and curcumin have a synergistic effect in the potentiation of G551D-CFTR (Yu, Miki et al. J Cystic Fibrosis 10: 243 - 252, 2011). However, the mechanism through which these compounds increase the CFTR activity is still unclear.

To study the mechanisms of genistein and curcumin, we investigated the effects of genistein and curcumin on the non ATP-hydrolytic CFTR mutants, K1250A- and E1371S- CFTR, expressed in CHO cells using whole-cell clamp technique. The reflect to the single channel currents. Because of their open probability close to 1, the whole-cell currents are thought to reflect the amplitude of their single channel currents.

Curcumin did not significantly affect the whole-cell currents obtained from CHO cells expressing K1250A- or E1371 S-CFTR whereas genistein induced a voltage-dependent block on them. Interestingly a combined application of genistein and curcumin induced a voltage-independent reversible reduction in K1250A- or E1371A-CFTR whole-cell currents. This current reduction seemed to be mainly induced by the genistein and curcumin accessing to CFTR proteins from the external side.

2788-Pos Board B558

Evolutionary and Mechanistic Insights into ABC Exporters

Attila Gulyas-Kovacs, David C. Gadsby.
The Rockefeller University, New York, NY, USA.

A prevalent model holds that ABC transporter function involves an ATP-driven conformational cycle in which ATP hydrolysis dissociates a tight dimer of nucleotide binding domains (NBDs), so propelling the transmembrane domain (TMD) conformation from outward to inward facing. Detailed characterization of these TMD events lags behind those in the NBDs because the TMDs are structurally diverse and more refractory to structural analysis. Recently we developed a bioinformatic approach that predicts evolutionarily conserved interactions between pairs of sequence positions. Like other approaches, ours gauges how, at each position, amino acid variation across aligned homologous sequences correlates with that at any other position. Distinctively, our approach exploits structural input to optimize performance through a side-chain contact prediction test. We applied this approach separately to the ABCB and ABCC subfamilies (represented by Pgp and CFTR) that are clearly, albeit distantly, homologous for all domains. Their TMD dimers contain 2x6 transmembrane helices in distinct bundles: two 'wings' at the extracellular side and two pairs of intracellular 'loop' (ICL) extensions. Comparing inward to outward facing structures suggests that rigid body motions of these bundles underlie transport mechanism. In our bioinformatic analysis, the precise pattern of predicted position pairs differed between subfamilies, alluding to their deep evolutionary segregation. But some general patterns were shared: pairs separated by one helical turn, and those between helices of the same bundle, were frequently predicted and may provide stability and rigidity to bundles throughout the transport cycle. On the other hand, a few predicted pairs between bundles exhibited strikingly different spatial separation in opposing conformations, such as Q179-V260 (ICL1-ICL2), separated by 5.7 Angstroms in CFTR modeled in the outward conformation but 16.1 Angstroms in the inward conformation. Thus, this approach provides detailed evolutionary and mechanistic insights into large classes of ABC exporters. [DK51767].

2789-Pos Board B559

Voltage-Dependent Gating of ClC-2 Chloride Channel

Jorge E. Sanchez-Rodriguez[1], Juan A. Contreras-Vite[2],
Pablo G. Nieto-Delgado[1], Alejandra Castro-Chong[1],
Jose A. De Santiago-Castillo[2], **Jorge Arreola**[1].
[1]Univ. Autonoma de San Luis Potosi, San Luis Potosi, Mexico,
[2]Univ. Michoacana de San Nicolas de Hidalgo, Morelia, Mexico.

The gating of ClC-2 Cl^- channel is facilitated by elevated $[H^+]_e$ and $[Cl^-]_i$ by interacting with the protopore gate whilst $[Cl^-]_e$ had not effect. In contrast, the gating of ClC-0 Cl^- channel is facilitated by protonation of the protopore gate by intracellular $[H^+]_i$ in a manner that is dependent on the extracellular $[Cl^-]_e$. To gain insights into the V_m dependent mechanism of ClC-2 expressed in HEK cells, we determine the V_m-dependence of open probability ($P_A(V_m)$) at different $[H^+]_i$, $[H^+]_e$ and $[Cl^-]_i$ using the patch clamp technique. Changing $[H^+]_i$ by 5 orders of magnitude whilst $[Cl^-]_i/[Cl^-]_e$=140/140 or 10/140 mM did not altered the onset kinetics but channel closing became faster at acidic pH_i and $P_A(V_m)$ curves were shifted towards more negative V_m. These results suggest that $[H^+]_i$ did not facilitated gating. In contrast, a same change in $[H^+]_o$ with $[Cl^-]_i/[Cl^-]_e$=140/140 mM enhanced P_A in a bi-phasic manner and shifted $P_A(V_m)$ curves to positive V_m. Importantly, P_A was >0 with $[H^+]_o=10^{-10}$ M and channel closed more slowly when $[H^+]_o$ or $[Cl^-]_i$ increased. This implied that ClC-2 can be gated without protonation and that external H^+ and/or internal Cl^- stabilized the open state. A kinetic analysis of Cl^- currents and $P_A(V_m)$ curves at different $[H^+]_o$ and $[Cl^-]_i$ using a gating scheme coupled to Cl^- permeation indicated that protonation of the protopore gate has negligible V_m- and Cl^--dependence and that the rate constant for closed-open transition of un-protonated channels were facilitated by elevated $[Cl^-]_i$ in a V_m-dependent manner. We propose that the majority of the V_m-dependence is due to a V_m-dependent occupancy of ClC-2 pore by the permeant Cl^- and that the open conformation is stabilized by a V_m-independent protonation and the Cl^- occupancy. Supported by CONACyT.

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Alkaline pH Block of CLC-K Kidney Chloride Channels Mediated by a Pore Lysine Residue

Antonella Gradogna, Michael Pusch.
Istituto di Biofisica, Genoa, Italy.

The human chloride channels ClC-Ka/Kb, as their murine orthologues ClC-K1/K2, are expressed in kidney and inner ear epithelia where they are involved in NaCl reabsorption and endolymph production, respectively. Mutations in ClC-Kb and barttin, an essential CLC-K channel beta subunit, lead to Bartter syndrome. Recently we identified the external residue H497 responsible for block of ClC-Ka at acid pH (Gradogna et al. 2010. J Gen Physiol 136:311). Now we